

## Claims:

1. A serine recombinase comprising a catalytic domain and a DNA binding domain wherein said catalytic domain is mutated at G101 or at a position corresponding to G101 of Tn3 resolvase.
2. A serine recombinase according to claim 1 wherein the mutation is G101S.
3. A serine recombinase comprising a catalytic domain and a DNA binding domain wherein said catalytic domain is mutated at Q105 or at a position corresponding to Q105 of Tn3 resolvase.
4. A serine recombinase according to claim 3 wherein the mutation is Q105L.
5. A serine recombinase comprising a catalytic domain and a DNA binding domain wherein said catalytic domain is mutated at D102 or at a position corresponding to D102 of Tn3 resolvase, and wherein the serine recombinase is not a D102Y E124Q mutant.
6. A serine recombinase according to claim 3 wherein the mutation is selected from D102Y, D102I, D102F, D102T, D102V, D102W or D102A.
7. A serine recombinase according to any one of the preceding claims further comprising one or more additional mutations selected from the group L105Q, V107M, V107L,

V107F, Q105L, A117V, R121K, E124Q, E124A, A89T, F92S, M103I or at positions corresponding to these mutations in Tn3 resolvase.

8. A serine recombinase according to any one of the preceding claims further comprising a one or more mutations of the surface residues corresponding to a '2,3' interface.

9. A serine recombinase according to claim 8 wherein the one or more mutations of the surface residues corresponding to a '2,3' interface include R2A and E56K or positions corresponding to R2A and E56K in Tn3 resolvase.

10. A serine recombinase according to any one of the preceding claims further comprising a one or more mutations of the surface residues corresponding to a '1,2' interface.

11. A serine recombinase according to claim 10 wherein the one or more mutations of the surface residues corresponding to a '1,2' interface include L66, G70, M76, M103, V107, T109, A117, R121, and E124 or positions corresponding to L66, G70, M76, M103, V107, T109, A117, R121, and E124 in Tn3 resolvase.

12. A serine recombinase according to any one of claims 1-9 further comprising the mutations R2A, E56K, G101S, D102Y, M103I and Q105L or the positions corresponding to these mutations in Tn3 resolvase.

13. A serine recombinase according to claim 12 further comprising the mutation V107F or the position corresponding to this mutation in Tn3 resolvase.

14. A serine recombinase according to any one of the preceding claims which is selected from the group consisting of Tn3 resolvase, Sin recombinase,  $\gamma\delta$  resolvase, Tn 21 resolvase,  $\beta$  resolvase, ISXc5 resolvase, Gin resolvase, Hin resolvase, Methanococcus jannaschii.resolvase, IS607 resolvase, ccrA1 resolvase, TN4451 resolvase, TP901-1 resolvase and  $\phi$ C31 resolvase.

15. A nucleic acid sequence encoding a serine recombinase according to any one of the preceding claims.

16. A nucleic acid expression vector comprising a nucleic acid sequence according to claim 15.

17. A host cell comprising a nucleic acid sequence according to claim 15 or a nucleic acid expression vector according to claim 16.

18. A hybrid recombinase comprising a catalytic domain from a serine recombinase connected by way of a linker to a heterologous DNA binding domain wherein said hybrid recombinase is capable of binding nucleic acid by way of said DNA binding domain and said catalysing recombination of said DNA.

19. A hybrid recombinase according to claim 18 wherein the heterologous DNA binding domain is the DNA binding domain of Zif268.

20. A hybrid recombinase according to claim 19 wherein the Zif268 DNA binding domain comprises a wild-type sequence starting from residue 2.

21. A hybrid recombinase according to claim 18 wherein the Zif268 DNA binding domain is mutated at one or more amino acids.

22. A hybrid recombinase according to any one of claims 18 to 21 wherein the catalytic domain is mutated at G101 or at a position corresponding to G101 of Tn3 resolvase.

23. A hybrid recombinase according to claim 22 wherein the mutation is G101S.

24. A hybrid recombinase according to any one of claims 18 to 21 wherein said catalytic domain is mutated at Q105 or at a position corresponding to Q105 of Tn3 resolvase.

25. A hybrid recombinase according to claim 24 wherein the mutation is V107F.

26. A hybrid recombinase according to any one of claims 18 to 21 wherein said catalytic domain is mutated at D102 or at a position corresponding to D102 of Tn3 resolvase.

27. A hybrid recombinase according to claim 26 wherein the mutation is selected from D102Y, D102I, D102F, D102T, D102V, D102W or D102A.

28. A hybrid recombinase according to any one of claims 18 to 27 wherein said catalytic domain comprises one or more additional mutations selected from the group R2A, E56K, G101S, D102Y, L105Q, V107M, V107L, V107F, Q105L, A117V, R121K, E124Q, E124A, A89T, F92S, M103I or at position corresponding to these mutations in Tn3 resolvase.

29. A hybrid recombinase according to claim 28 wherein said catalytic domain comprises the mutations R2A, E56K, G101S, D102Y, M103I and Q105L or the positions corresponding to these mutations in Tn3 resolvase.

30. A hybrid recombinase according to claim 29 further comprising the mutation V107F or the position corresponding to this mutation in Tn3 resolvase.

31. A hybrid recombinase according to any of one of claims 18 to 30 wherein the catalytic domain is between 125 and 146 amino acids in length.

32. A hybrid recombinase according to claim 31 wherein said catalytic domain is 125 amino acids in length.

33. A hybrid recombinase according to claim 31 wherein the catalytic domain is 146 amino acids in length.

34. A hybrid recombinase according to claim 31 wherein the catalytic domain is 140 amino acids in length.

35. A hybrid recombinase according to claim 31 wherein the catalytic domain is 144 amino acids in length.

36. A hybrid recombinase according to any one of claims 18 to 35 wherein the linker sequence is selected from the group consisting of TVDRSSDPTSQ, GSGGSG, GSGGSGGSG, GSGGSGGSGGSG, GGGSGGG, GGGSGGGGSGGG, TVDRSSDPTSQTS, GSGGSGTS, GSGGSGGSGTS, GSGGSGGSGGSGTS, GGGSGGGTS, GGGSGGGGSGGGTS, NRVAQQLAGKQS, SDYTQNNIHO, TVDRTS and TS.

37. A hybrid recombinase according to claim 36 wherein the linker sequence is TVDRTS.

38. A hybrid recombinase according to any one of claims 18 to 37 wherein the catalytic domain is a Tn3 resolvase catalytic domain.

39. A hybrid recombinase comprising a Tn3 resolvase catalytic domain, which catalytic domain comprises the mutations R2A, E56K, G101S, D102Y, M103I and Q105L and V107F, linked to a DNA binding domain via a linker comprising the sequence TS, wherein said hybrid recombinase is capable of binding nucleic acid by way of said DNA binding domain and catalysing recombination of said DNA.

40. A hybrid recombinase according to claim 39 wherein the linker comprises the sequence TVDRTS.

41. A hybrid recombinase according to claim 39 wherein the catalytic domain is amino acids 1 to 148 of a TN3 resolvase catalytic domain.

42. A hybrid recombinase according to claim 39 or claim 40 wherein the catalytic domain is amino acids 1 to 144 of a TN3 resolvase catalytic domain.

43. A nucleic acid sequence encoding a hybrid recombinase according to any one of claims 18 to 42.

44. A nucleic acid expression vector comprising a nucleic acid sequence according to claim 43.

45. A host cell comprising a nucleic acid sequence according to claim 43 or a nucleic acid expression vector according to claim 44.

46. A catalytic domain of a serine recombinase which has been mutated at G101 or at a position corresponding to G101 of Tn3 resolvase.

47. A catalytic domain according to claim 46 wherein the mutation is G101S.

48. A catalytic domain of a serine recombinase which has been mutated at Q105 or at a position corresponding to Q105 of Tn3 resolvase.

49. A catalytic domain according to claim 48 wherein the mutation is Q105L.

50. A catalytic domain of a serine recombinase which is mutated at D102 or at a position corresponding to D102 of Tn3 resolvase, and wherein the catalytic domain does not further comprise a mutation at E124Q.

51. A catalytic domain according to claim 50 wherein the mutation is selected from D102Y, D102I, D102F, D102T, D102V, D102W or D102A.

52. A catalytic domain according to any one of claims 46 to 51 further comprising one or more additional mutations selected from the group L105Q, V107M, V107L, V107F, Q105L, A117V, R121K, E124Q, E124A, A89T, F92S, M103I or at positions corresponding to these mutations in Tn3 resolvase.

53. A catalytic domain according to any one of claims 46 to 52 further comprising a one or more mutations of the surface residues corresponding to a '2,3' interface.

54. A catalytic domain according to claim 53 wherein the one or more mutations of the surface residues corresponding to a '2,3' interface include R2A and E56K or positions corresponding to R2A and E56K in Tn3 resolvase.

55. A catalytic domain according to any one of claims 46 to 54 further comprising a one or more mutations of the surface residues corresponding to a '1,2' interface.



56. A catalytic domain according to claim 55 wherein the one or more mutations of the surface residues corresponding to a '1,2' interface include L66, G70, M76, M103, V107, T109, A117, R121, and E124 or positions corresponding to L66, G70, M76, M103, V107, T109, A117, R121, and E124 in Tn3 resolvase.

57. A catalytic domain according to any one of claims 46 to 56 further comprising the mutations R2A, E56K, G101S, D102Y, M103I and Q105L or the positions corresponding to these mutations in Tn3 resolvase.

58. A catalytic domain according to claim 57 further comprising the mutation V107F or the position corresponding to this mutation in Tn3 resolvase.

59. A catalytic domain according to any one of claims 46 to 58 which is selected from the group consisting of Tn3 resolvase, Sin recombinase,  $\gamma\delta$  resolvase, Tn 21 resolvase,  $\beta$  resolvase, ISXc5 resolvase, Gin resolvase, Hin resolvase, Methanococcus jannaschii.resolvase, IS607 resolvase, ccrA1 resolvase, TN4451 resolvase, TP901-1 resolvase and  $\Phi$ C31 resolvase.

60. A nucleic acid sequence encoding a catalytic domain of a serine recombinase according to any one of claims 46 to 59.

61. A nucleic acid expression vector comprising a nucleic acid sequence according to claim 60.

62. A host cell comprising a nucleic acid sequence according to claim 60 or a nucleic acid expression vector according to claim 61.

63. A method for identifying a hyperactive mutant serine recombinase capable of catalysing site-specific DNA recombination when bound to a recognition site comprising fewer nucleotides than necessary for achieving recombination with a corresponding wild-type serine recombinase, comprising the steps of

(a) mutating said wild-type serine recombinase such that the mutant recombinase comprises one or more mutations, in a catalytic domain of the recombinase, with respect to the wild-type serine recombinase; and

(b) detecting whether or not said mutant serine recombinase is capable of catalysing DNA recombination when bound to said recognition site comprising fewer nucleotides than necessary for achieving recombination with the corresponding wild-type serine recombinase

64. A method of recombining DNA comprising contacting a first DNA sequence and a second DNA sequence with a serine recombinase according to any one of claims 1 to 14 under suitable conditions for allowing a recombination of said first and second DNA sequences.

65. A method of recombining DNA comprising contacting a first DNA sequence and a second DNA sequence with a serine recombinase according to any one of claims 18 to 40 under suitable conditions for allowing a recombination of said first and second DNA sequences.

66. A method according to claim 64 or claim 65 wherein said first DNA sequence and said second DNA sequence comprise at least the 28bp binding site I of Tn3 resolvase.

67. A kit for recombining a first DNA sequence and a second DNA sequence, said kit comprising a serine recombinase according to any one of claims 1 to 14.

68. A kit for recombining a first DNA sequence and a second DNA sequence, said kit comprising a hybrid recombinase according to any one of claims 18 to 42.

69. A kit for recombining a first DNA sequence and a second DNA sequence, said kit comprising any one or more of the following

- i a nucleic acid sequence according to claim 15;
- ii a nucleic acid sequence according to claim 43;
- iii a nucleic acid sequence according to claim 60;
- iv a nucleic acid expression vector according to claim 16;
- v a nucleic acid expression vector according to claim 44; or
- vi a nucleic acid expression vector according to claim 61.